IN THE U.S. PATENT & TRADEMARK OFFICE

Applicants:	Shozo KOYAMA et al.		
Serial No.:	10/786,369	Group:	1641
Filed:	Feb 26, 2004	Examiner:	Shafiqul Haq
For:	METHOD FOR PRODUCING AN ANTIGENIC SUBSTANCE AND ANTIBODY		

DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents and Trademarks

Washington, D.C., 20231

Sir:

I, Shozo KOYAMA, a nation of Japan, residing at 48-2, Oazasatoyamabe, Matsumoto-shi, Nagano 390-02, Japan, do hereby declare as follows:

I am a co-applicant of the invention as described and claimed in the specification of the above-identified application.

I am familiar with the Office Action dated June 25, 2009, in which claims 35 and 37 are rejected as failing to comply with the written description requirement.

To show that cancer cells treated with Yoshixol are more effective for treating cancer than cancer cells treated with a lysis buffer (surfactant) or treated by homogenizer, I carried out the experiments described below.

Experiments

Method:

I) Mouse B16 melanoma cells were cultured in a medium supplemented with 10% fetal bovine serum at 37°C under 5% CO_2. B16 cells grown to 80% confluency were collected by trypsin treatment and suspended in a culture medium at a concentration of 2×10^6 cells/mL. The cell suspensions were subjected to treatment A, B or C below to obtain Solution A, B or C, respectively. The obtained

solutions A to C were filtered through 0.25 μm Millipore filter, and then the filtrates were centrifuged at 6000 rpm for 20 min. The 1.5 mL aliquot of each lower layer was recovered as a composition for administration to mice (Cell Compositions A, B and C).

A) The above-described cell suspension (5 mL) was homogenized with a homogenizer (Mini Cordless Grinder, Funakoshi Co.) for about 10 min to obtain Solution A.

B) To 5 mL of the above-described cell suspension, 5% CHAPS buffer (150mM NaCl, 1mM KCl, 10mM Tris-HCl, 1.2mM PMSF, pH8.0) as a lysis buffer was added to lyse cells to obtain Solution B.

C) To 5 mL of the above-described cell suspension, 0.1 mM of Yoshixol was added and the resulting mixture was incubated in a incubator (37°C, 5% CO_2) for 1 hour to obtain Solution C.

The scheme is shown in Fig. 1 below.

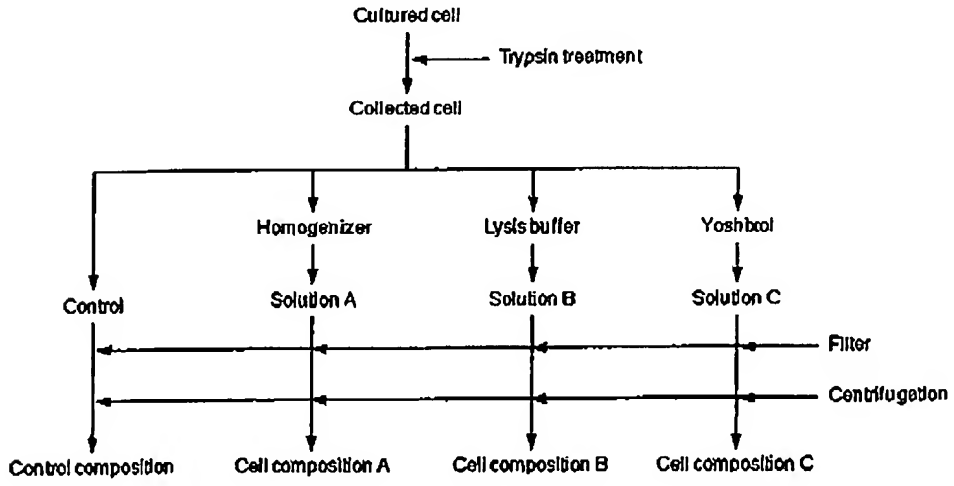

Fig. 1

II) To 6-week-old C57BL/6 mice (experimental group: Control, Homogenate, Lysate and Yoshixol; each group consisted of 5 mice), the above-prepared control

composition or Cell Composition A, B or C was subcutaneously administered at an amount of 0.1 mL per mouse twice at 1-week intervals. Immediately after 2nd administration, separately prepared B16 cells (50μL, 2 x 10^5) were intraperitoneally inoculated to each mouse of each group. At a month after the transplantation, mice were sacrificed to isolate the tumors in the abdominal cavity, and the tumors were weighed to assess the growth of the transplanted melanoma cells.

Fig. 2 shows the representative state of the transplanted melanoma cells in the abdominal cavity in each group. In Control, Homogenate and Lysate group, transplanted melanoma cells grew to form a lot of black tumors. On the other hand, in Yoshixol group, growth of the melanoma cells was dramatically inhibited.

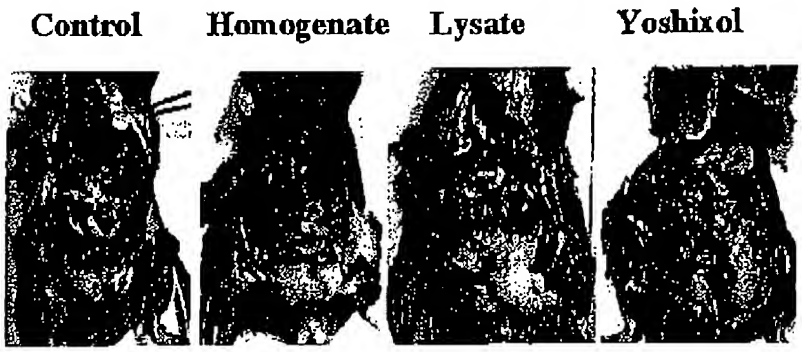

Fig. 2

Fig. 3 shows the weight of the isolated tumor in each group. There was no significant difference in growth of the tumor between Control and Homogenate group. The tumor growth in Lysate group and Yoshixol group was significantly inhibited when compared to Control group. The prominent inhibitory effect was observed in Yoshixol group. These results indicate that cell sediment obtained by treating cancer cells with Yoshixol comprises component(s) useful as a cancer vaccine.

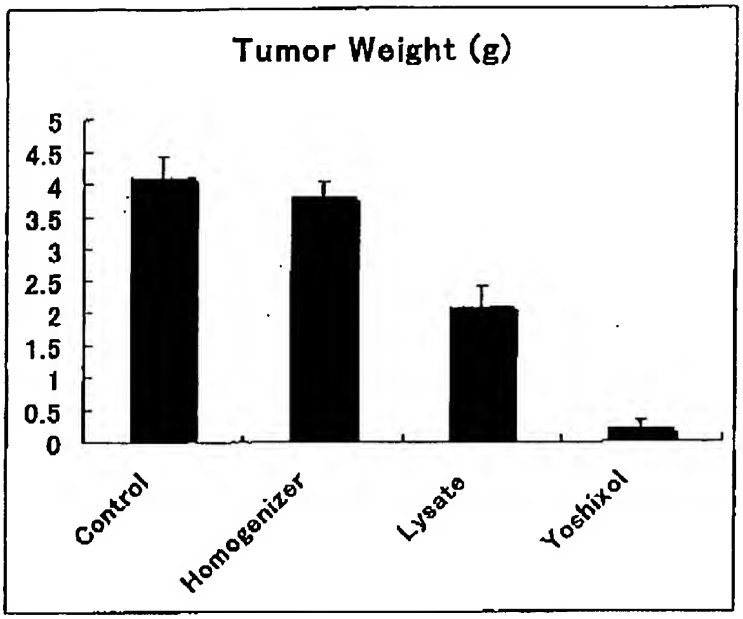

Fig. 3

These results also seem to show that Yoshixol can kill cells while sustaining a functional cellular structure such as protein structure. When cells are mechanically destroyed by a homogenizer treatment, proteins are fragmented to lose their functional structures. This change is shown in Fig. 4 below. Fig. 4 shows the results of the two-dimensional electrophoresis of retina tissue samples with or without mechanical (homozenizer) treatment. Unnatural spots (x) of fragmented proteins were observed only in a mechanically destroyed sample (Fig. 4, "Homogenized")

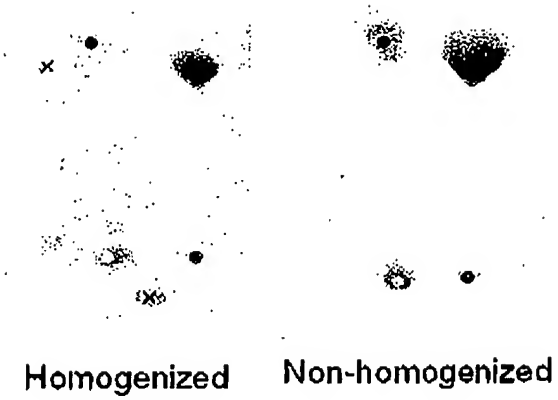

Fig. 4

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 10th day of September, 2009

Shozo KOYAMA